ENHANCING VALUE AND USE OF AGRICULTURAL AND FOREST PRODUCTS

The Enhancing Value and Use of Agricultural and Forest Products Division responds to the growing need to enhance the competitive value and quality of U.S. agricultural and forest products. Research in this area builds the scientific base of knowledge to use agricultural and forest materials more fully and effectively. The Division supports both fundamental and applied research on new and improved processes and on development of new uses for agricultural and forest materials. Program Areas in this Division include: Value-Added Products Research encompassing Food and Non-Food Characterization/Process/ Product Research, and Improved Utilization of Wood and Wood Fiber.

FOOD CHARACTERIZATION/PROCESS/PRODUCT RESEARCH

Panel Manager - Dr. E. Allen Foegeding, North Carolina State University
Program Director - Mr. Jeffery L. Conrad

This program area seeks to better understand the properties (physical, chemical and biological) of raw agricultural materials and products related to their quality and processing characteristics and to develop innovative products and processes for better utilization and more efficient conversion of agricultural materials to higher value food products. Specifically, research is supported on value-added food products which contribute to expanded markets for agricultural commodities, lower-cost food products, and a more competitive domestic food industry with expanded export opportunities. This program supports research to increase the quality, utility, convenience, nutrient value, and safety of food products through innovative processing methods. Research providing the basis for development of new food products is also supported

9801844 Detection of Bone Fragments in Poultry Meat Using Combined X-ray and Laser Imaging
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Strengthening Award; Grant 98-35503-6605; $120,000; 3 Years

With the United States producing over 13.2 billion pounds of boneless chicken meat annually and consumers increasingly demanding safe, high quality boneless meat at a low cost, the accurate and efficient detection of bone fragments and other hazards in poultry meat has great urgency. Current x-ray technology addressing these problems has very limited success (over 30% detection errors) mainly due to its inability to consistently recognize bone fragments in meat of uneven thickness. This project will develop a technology that integrates x-ray imaging with laser 3-D imaging to compensate for the uneven thickness of poultry meat and to enhance the x-ray accuracy in bone fragment detection and augment the inspection system's capability to rapidly and accurately detect bone fragments or hazards. Our preliminary studies indicate the promise and feasibility of this technology.

This research will be conducted in three stages. First, we will determine x-ray absorption coefficients and specify optimal settings for bone fragment detection. Next, we will develop a high resolution laser 3-D imaging system, determining variations in thickness of poultry fillets at real-time. Finally, we will combine x-ray and laser 3-D imaging techniques, producing integrated images, developing image processing algorithms for bone fragment recognition. The results from this study will greatly advance the x-ray technology in food inspection and promise to improve the quality, safety, and cost-effectiveness of U.S. poultry products.

9801191 Postmortem Changes in the Calpain System and Their Relationship to Tenderness.
Goll, D. E.
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Grant 98-35503-6324; $165,000; 3 Years

This project will attempt to learn how the calpain system functions to increase meat tenderness during postmortem storage of muscle tissue. It is well-known that meat becomes increasingly tender if it is left to "age" after slaughter, and steaks sold in expensive restaurants are invariably obtained from carcases that have been aged for three weeks or longer. This aging process is very expensive, however, because it requires refrigerated space that is costly to maintain. A large amount of experimental evidence has accumulated during the last 10-15 years showing that two proteolytic enzymes called micromolar and millimolar calpain (μ-calpain and m-calpain) are responsible for almost all the proteolysis associated with the increased tenderness during postmortem aging. The ability of these two proteolytic enzymes to degrade muscle proteins and increase tenderness is inhibited by a third protein, called calpastatin, that also exists in muscle tissue. Together, these three proteins constitute the calpain system. The evidence acquired during the past 10-15 years indicates that ability of calpastatin to inhibit the calpains is very highly related to rate of postmortem tenderization. Because skeletal muscle contains enough calpastatin to inhibit all calpain activity in its cells, it is not clear why higher or additional calpastatin activity should cause further inhibition of calpain proteolysis in postmortem muscle. This project seeks to determine how calpain activity is controlled in postmortem muscle, and whether this activity can be manipulated to increase the rate of postmortem tenderization. The ability to alter calpain activity in postmortem muscle would greatly decrease the aging time required to produce uniformly tender meat, and should increase consumer satisfaction.

9801319 A Conjugal System for Genetic Delivery and Alleic Replacement in Lactic Acid Bacteria
Mills, D.
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New Investigator Award; Grant 98-35503-6989; $101,700; 2 Years

Lactic acid bacteria (LAB) are commonly used as starter cultures in the production of a variety of fermented foods. Recent advances in molecular biology have permitted a wide array of genetic analysis of these commercially important bacteria. Unfortunately,

molecular analysis of many LAB remains problematic due to lack of viable gene transfer and gene replacement systems. The conjugative element pRS01, from *Lactococcus lactis* ML3 is known to transfer at high frequencies to other Lactococcal recipients. Moreover, pRS01 has been shown to transfer to a wide range of LAB genera including, lactococci, streptococci, leuconostoc, enterococci, and lactobacilli. Recently the origin of transfer (*oriT*) of pRS01 was identified. Non-conjugative plasmids harboring the cloned *oriT* from pRS01 were shown to be mobilized by pRS0I into other lactic acid bacteria such as leuconosotoc, enterococci and lactobacilli. This proposal will focus on the development of appropriate genetic constructs to enable conjugative delivery of novel genetic material into various LAB. First, the host range for delivery of various *oriT*-containing plasmid vectors will be examined with a focus on those LAB recipients that are currently resistant to alternative transformation methods. Second additional *oriT*-containing vectors will be examined for their ability to promote allelic exchange within *Lactococcus lactis* subsp. *cremoris* by deletion of the temperate bacteriophage rlt. Finally, the *oriT*-driven allelic exchange system will be assessed in other lactic acid bacterial genera by interruption of the host gene(s) encoding lactate dehydrogenase. A viable genetic system for the LAB will aid starter culture development for a variety of fermented foods.

9801542 New Approaches to Improve Quality and Safety in Cooking of Hamburger Patties

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Grant 98-35503-6453; $125,000; 2 Years

Hamburgers are the fastest growing food items consumed in the United States. Americans consumed 6 million more hamburgers in a 2-week period in 1996 than they did in the same 2-week period in 1995. Furthermore, U.S. companies benefit from the worldwide popularity of hamburgers. To manufacture safe, high-quality products and introduce processing innovations in the prepared-food industry, a fundamental understanding of the cooking process is required. The textural quality of cooked hamburger patties can be improved and the safety risk from undercooking can be minimized by (a) obtaining a fundamental description of heat and mass transfer, (b) quantifying changes in physical properties, and (c) determining microbial lethality during the cooking process. An increased level of understanding of the material science (in our case hamburger meat) and use of predictive modelling (of the cooking process of patties) can provide improved recommendations for the design of new equipment. This can help alleviate mishaps associated with the survival of pathogens such as *E. coli* O157:H7 in undercooked patties.

The proposed research will specifically address the following objectives: (1) Determine changes in physical properties of hamburger patties as influenced by processing variables. (2) Develop predictive mathematical models to describe heat transfer in hamburger patties involving pathogen destruction. (3) Using optimization methods, develop new and improved cooking processes for hamburger patties that ensure a safe product with desirable levels of textural quality. The results of this study are anticipated to develop fundamental information for innovations in the prepared-food industry and ultimately benefit the consumer with an improved and safe product.

9801482 Quantification of Flavor Quality in Fresh Tomatoes

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Grant 98-35503-6383; $165,618; 3 Years

This project seeks to provide a better understanding of how composition of fresh tomatoes affects consumer acceptability. When we bite into a fresh tomato the sweet, sour and salty taste on our tongue is enhanced by aromatics which are released during chewing and passed through the back of our mouth to the nose. It is this combination of taste and aroma that leads to what we call flavor, and it is our reaction to what we perceive that we call quality. As we chew a fresh tomato we can observe many different sensations. Using a trained sensory panel, this project will describe flavor for a large number of commercial varieties and breeding lines on the basis of the different sensations produced during chewing with particular emphasis on the differences in the aromatics. Using an electronic nose it will attempt to classify these varieties and breeding lines into those with superior, acceptable and unacceptable flavors. Chromatographic separation techniques will be used to relate specific sensory sensations to the natural chemicals in the tomato responsible for aroma. These chemicals will in turn be linked directly to the natural tomato enzymes that produce them and the genes responsible for the differences between the varieties and lines studied. By integrating very different scientific approaches we anticipate development of a consumer definition of fresh tomato flavor and establishment of key genetic, biochemical and chemical factors that influence its perception by our nose.

9801183 Developing and Characterizing Exotic Corn Crosses with Value-Added Starch and Oil

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Grant 98-34503-6371; $155,000; 3 Years

Our long-term goal is to develop corn inbred lines with value-added starch and oil properties that will enhance the competitive value of U.S. agricultural products. The goal of this particular application is to target five corn lines for further development and for

extensive starch structural and functional analyses. Our preliminary analyses of their thermal properties shows unusual starch functions for these lines. Also, we will screen additional lines for potential valuable traits in their starch and oil components.

The general goals of this work will be accomplished through these specific research objectives: (1) Define the primary chemical starch structure in the five corn lines that have been verified to possess unique thermal properties; (2) Evaluate starch function in the five corn lines referenced in Objective # 1 that have been verified to possess unique thermal properties; and (3) Continue efforts to seek exotic Corn Belt lines with unique starch functions and fatty acid compositions.

Corn lines selected for this study originated from a program designed to rescue and utilize irreplaceable corn germplasm in 12 countries. These diverse, elite accessions of corn were selected from 12,000 lines native to Latin America. The rationale for focusing this application on the development of starch and oil from these alternate sources of corn is that: (1) the use of corn from underutilized sources offers extensive variety in raw material selection, and (2) once valuable oil and starch components are identified, their use will expand the markets for corn. In addition, we hypothesize that starches with proven unique thermal properties will have functional properties of great industrial value. The proposed research is significant, because it can be expected to have an important positive impact on plant agriculture. The value-added materials being developed will contribute to U.S. crop diversification, will expand domestic and international markets for agricultural commodities, will promote environmentally sound manufacturing processes, will enhance rural economies involved in specialty grain production and will return higher prices per acre so small farms can remain viable.

9801239 Mechanisms for Shift of Native Enzyme Plasmin from Casein to Whey Fraction of Bovine Milk

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Grant 98-35503-6370; $170,000; 3 Years

Proteins in foods can be broken down by enzymes called proteases to cause desirable or undesirable flavor and texture changes. Plasmin, the major native protease in milk, is associated with the casein fraction of milk. We have shown that when cold-loving microorganisms (i.e., psychrotrophs) grow during refrigerated storage of milk prior to processing, plasmin is released from the casein micelle of milk into the whey fraction. This shifting of plasmin activity has serious implications for the quality of certain dairy products. Plasmin plays a predominant role in the ripening of some cheese, so plasmin activity in the casein curd is desirable. However, plasmin is generally undesirable in dry casein and whey protein products, which are used widely as ingredients in many food products because of their excellent functional properties. High plasmin levels in casein or whey protein products could cause undesirable protein breakdown in food systems to which they are added. The specific objectives of the research proposed are to determine the mechanisms by which plasmin activity: (1) is released from casein micelles into whey, due to bacterial proteases and due to the enzyme and acid treatments used in cheesemaking, and (2) is decreased in whey after the initial increase. Results of these experiments will enhance our understanding of plasmin system regulation in milk and make it possible to recommend conditions that can be used to either enhance or minimize plasmin activity in the casein and whey fractions, depending on the application. This knowledge and the recommendations made have great potential for improving the quality and reducing the production costs of certain dairy products and making our domestic producers of milk protein products more competitive.

9801527 Objective Characterization of Texture Properties of Crunchy Foods During Chewing

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Grant 98-35503-6328; $120,000; 2 Years

Texture properties of crunchy foods, such as hardness, fracturability, crispness, cohesiveness, and adhesiveness have been considered difficult to evaluate using instrumental approaches. Those properties are affected not only by food ingredients and processing conditions, but also by the interactions between food components and saliva during chewing.

Chewing causes many facial motions. These motions include masseter muscle contraction, digastric and temporalis muscle motions, and joint and jaw movements. Those special points respond differently in chewing different foods in the manner of either intensity or frequency or their distributions. The purpose of this research is to validate an objective method that will use an electronic sensing system to observe and quantify texture properties of crunchy foods during chewing. Specific objectives are (1) to relate textural sensory attributes with extracted features from the recorded electronic sensing signals obtained during chewing; (2) to develop and simplify standard testing procedures and control variables; and (3) to develop prototypical testing systems that could be used by cooperating companies interested in evaluating crunchy foods.

The information and techniques developed from this research will be useful for studying texture properties of food products during the product development stage, during food storage, and for food product quality improvement. The system developed could be a powerful tool for sensory scientists to study sensory and texture properties and gain a better understanding of sensory processes.

9801188 Light Backscatter Sensor Development for Measurement of Food Consistency

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Grant 98-35503-6452; $180,000; 3 Years

Further automation is needed in U.S. food processing facilities not only to maintain competitiveness in the world economy but also to improve product consistency, quality, and safety. The lack of suitable sensors for characterizing the properties of liquid particulate food materials is hindering the implementation of modern process control technologies. Improved sensors and their resulting process control benefits will allow for tighter production tolerances, increased consistency of food properties, process optimization, improved quality, and savings in raw materials, energy, and waste disposal. As the benefits of in-line process control are realized the need for precise, in-line monitoring systems capable of providing tight control of critical parameters will increase. The overall objective of the proposed work is to develop a versatile, reliable, simple sensor capable of monitoring important food processing parameters of liquid particulate process fluids. The proposed fiber optic sensor is based on the measurement of light scattering and its relationship to the physical properties of the sample. Before advanced optical sensors can be designed, detailed knowledge of the functional relationship of light propagation through the particulate media, such as milk, is necessary. In this work, the relationship between milkfat and casein contents and the resulting light scattering of milk will be determined. Based on this, an optimized sensor for milk processing applications will be designed and fabricated. To realize the full potential and versatility of such a sensor, studies will be performed to investigate the use of the sensor with additional food products, such as peanut butter, mayonnaise, ketchup, mustard, etc.

9801526 Extraction, Purification and Therapeutic Evaluation of γ-oryzanol Components of Rice Bran

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Grant 98-35503-6327; $120,000; 2 Years

Rice bran is an abundant source of several compounds that may have important nutritional or pharmacological properties. Most abundant of these (0.5% of rice bran) is a class of compounds referred to as γ-oryzanol, which has been suggested to have the ability to lower serum cholesterol and/or prevent certain types of cancer. It is also possible that individual components of γ-oryzanol (sterols and phenolic antioxidant derivatives) could be even more powerful in this regard, if they could be purified from the rice bran. The specific aim of this research will be to extract, purify and evaluate the potential of individual components of γ-oryzanol as pharmaceutical products. We will utilize a specially designed extraction technique that uses CO_2 under high pressure and elevated temperature (referred to as super critical CO_2) that improves the yield of these compounds from rice bran. The compounds will be purified using large scale high performance liquid chromatography and screened for activity using cell cultures. Ultimately, the most promising components could be identified and commercial processes developed to exploit them as a value-added co-product of rice processing. In view of the quantity of rice that is processed world-wide, increasing the value of rice bran, currently a waste product of the rice processing industry, would have tremendous economic significance.

9801786 Production of Acetylated Glycerides in Transgenic Oil Crops

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Grant 98-35503-6326; $185,000; 3 Years

Vegetable oils are a major agricultural commodity with U.S. production generating about $5-6 billion in oil sales revenues. Triacylglycerols constitute >98% by weight of vegetable oils. Their synthesis has been extensively studied in seeds and many genes for enzymes of fatty acid and lipid synthesis have been cloned. However, the only enzyme unique to triacylglycerol biosynthesis, and the very last enzymatic step in the pathway, acyl-CoA:1,2-diacylglycerol *sn*-3-acyltransferase (DAGAT), has been retractable to purification and no gene has been reported cloned from any organism. Using a novel seed system that makes triacylglycerols with a reduced carbon number we have developed a unique purification strategy that we believe will lead to the purified enzyme, and hence to the gene. The purification of this DAGAT protein, the cloning of the gene and a study of gene expression in seeds are the primary objectives of this project. One result we will be looking for is whether over-expression will increase seed oil content, and ultimately oil yield per acre. Given the revenue stream from the US production of vegetable oils, a generic technology that increases the oil yield only 1% while leaving protein yield unaffected would be worth $50 million per annum if all the value could be captured. We anticipate that such a technology would be commercialized by the US seed business. The project should also furnish information on the production of structured acetyl glycerides through genetic engineering. Structured triacylglycerols are used in nutraceutical and reduced calorie applications of oils and fats.

9801477 Noninvasive Mapping of Heat and Mass Transfer During Immersion Frying

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Grant 98-35503-6382; $175,000; 3 Years

This proposal addresses two fundamental aspects of the frying process: (1) determination of mechanisms of oil uptake; and (2) quantification of heat flux at the oil/food interface. A mechanistic approach will be used to elucidate the driving forces involved in the movement of oil into a food during and after frying. We hypothesize that oil transport occurs in two periods, during frying and during cooling. While this has been proposed by other researchers it has not been completely tested as previous studies tested oil content of samples removed from the fryer thus rendering information regarding oil uptake during frying incomplete. Two recent studies have made significant advances in experimental techniques and have indicated that the majority of oil within a fried product is absorbed during the cooling phase (Ufheil and Escher, 1996; Moreira et al., 1997). Two laboratory methods will be employed in the proposed study, the use of single capillary tubes to study theoretical oil flux and the use of Magnetic Resonance Imaging (MRI) to determine oil flux and location during frying and post-fry cooling of Russet potatoes. This study will focus on determining the fundamental nature of oil transport and development of rigorous mathematical models will be used to complement laboratory findings.

Continued work on heat transfer will entail the mapping of heat flux at the solid/liquid interface. As described above, one of the critical components of the frying process is the high heat transfer rates. The ability to quantify these rates will aid in the development of alternative processes. Over the past year the lead investigator developed a method of determining heat flux and convective heat transfer coefficients during the boiling regime of frying. This is the first time a method has been developed to measure convective heat transfer coefficient for the boiling phase of frying. Study of convective heat transfer coefficient and changes in convective heat transfer coefficient due to process parameters such as oil temperature, oil degradation level, and product shape and orientation are necessary for continued advances by industry and will be addressed in the proposed research.

9801317 Thermal Transport In A Hot Air Jet Impingement Oven

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New Brunswick, NJ 08901-8520
Grant 98-35503-6355; $170,000; 3 Years

This project is aimed at the investigation of the transport phenomena associated with a hot air jet impingement oven, in which high speed jets of hot air are used to bake and toast food products such as breakfast cereals, cookies, breads, muffins, pizzas, burgers and roasted chicken or beef. Faster heating rates and lower cooking temperatures in such ovens lead to higher production rates and better product quality. The findings from this research will help food manufacturers and processors to predict and optimize the cooking process in hot air jet impingement ovens. In the proposed research, heat transfer characteristics between the jets of hot air and food products of different shapes and sizes will be investigated experimentally. Heat transfer rates and heat transfer coefficients will be measured using metal objects to represent foods of different shapes. Cylindrical object geometries will be chosen to represent products like hot dogs or sausages, and flat disk-like object geometries will be chosen to represent products like hamburgers, pizza or patties. The information obtained from food product models will be tested with real food products of similar geometries. The effects of oven conditions and object geometry on heat transfer characteristics will be investigated to develop the design rule essential for process optimization and scale-up.

9801304 Galacto-Oligosaccharide Production from Lactose by Immobilized -Galactosidase

Yang, S. T.
The Ohio State University; Department of Chemical Engineering; Columbus, OH 43210
Grant 98-35503-6325; $120,000; 2 Years

The goal of this project is to develop and study the feasibility of a novel enzymatic process to produce galacto-oligosaccharides (GOS) from whey lactose. The enzyme -galactosidase (lactase), commercially used to hydrolyze lactose in milk and whey, can also be used to produce oligosaccharides containing 2 to 5 galactose units and one glucose unit from lactose. These GOS are natural constituents in mother's milk. They have been found to efficiently and selectively accelerate the growth of Bifidobacteria in the lower intestine. These bacteria and GOS are known to have many beneficial effects on human health. In this project, we will develop an immobilized enzyme reactor to economically produce GOS from lactose. We will evaluate the feasibility of several novel approaches to enhance and to control GOS formation from lactose. A nanofiltration process will be used to separate the GOS present in the reactor product stream and recycle unreacted lactose; this will shift the reaction equilibrium towards GOS formation and increase the overall lactose conversion. Methods to increase the galactosyl transferase activity and inhibit hydrolytic activity of the enzyme will be studied with the goals of increasing GOS yield from current less than 40% (w/w) to more than 75% and reducing the product cost by ~50%. The proposed process would produce a high-value product (more than $20/lb) that can be used as a health-promoting food ingredient and dietary supplement from the surplus whey permeate and lactose (less than $0.4/lb) currently produced in the dairy industry. The market for GOS is at $200 million per year in Japan alone. The large, potential U.S. and worldwide markets should exceed $1 billion.

9801678 Debye Resonances of Polar Food Molecules Targeted for Uniform Capacitive Heating

Kolbe, E.; Flugstad, B.; Park, J. W.; Zhao, Y.
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Grant 98-35503-6338; $140,000; 2 Years

Growing concerns for food safety, coupled with market pressures for higher quality and value, indicate a need for better ways to pasteurize packaged foods. The ideal system would bring about a high internal temperature within a short period of time to destroy certain bacteria with minimum decrease of quality attributes such as color, moisture, texture. A multidisciplinary team will conduct research to bring about rapid and uniform dielectric capacitive heating in the radio frequency (RF) range, 2 orders of magnitude below commercial microwave frequencies. There are certain resonant ("Debye") frequencies at which maximum energy is absorbed by product components and packaging materials. We propose that frequency patterns and schedules could be selected to maximize product heating without burning the packaging material as products pass between shaped electrodes.

Focusing on commercial formulations of frankfurters and surimi seafoods, the project proposes: (1) To measure and characterize the dielectric properties, including Debye resonances, of various constituents of muscle foods and potential packaging materials, as functions of frequency (100 Hz - 100 MHZ) and temperature (0 - 90 ^{o}C); (2) To create models of dielectric properties, then to use these in existing computer software to guide the design and operation of full power tests; (3) To quantify effects of radio-frequency pasteurization on microbiological lethality, color, and texture; (4) To perform high energy capacitive heating experiments, testing effects of product and electrode geometry, field characteristics and time-variation on heating rates, pasteurization efficacy, and package integrity.

9801313 Improved Surimi Processing through Bioengineering of Proteinase Inhibitors

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Grant 98-35503-6384; $160,000; 3 Years

Surimi processing has provided a successful means of utilizing various underutilized marine species currently unsuitable for human consumption. These species contain high levels of a cysteine proteinase, which is extremely effective in degrading myosin; this proteinase's activity needs to be controlled during processing in order to avoid the loss of its quality as a functional ingredient. We have previously shown that use of cysteine proteinase inhibitors can effectively inhibit proteinases in fish muscle and have expressed the cloned rainbow trout cystatin cDNA as a fusion protein in *Escherichia coli*. The expressed rainbow trout cystatin C containing two disulfide bonds needs to be renatured to facilitate formation of proper disulfide bonds before exhibiting inhibitory activity against proteinases. The renaturation process requires harsh chemical treatments currently not acceptable in food applications. Soyacystatin has proven to be effective against a variety of cysteine proteinases tested, and it lacks disulfide bonds, therefore, offering a great advantage of high expression of active proteins and no requirement for the renaturation process of the produced protein. Both fish cystatin C and soyacystatin can be designed to improve inhibition efficiency by the newly developed phase display technique, which allows selection of mutant forms of the protein with desired traits, such as, higher inhibitory activity. The selected cystatins are expected to have increased inhibitory effect against proteinase and, thus, enhance gelation of surimi. The improvement of inhibitory function of cystatins will provide a greater economic benefit to seafood industry, and help sustain our valuable limited marine resources by more efficient utilization.

9801543 Classifiers for Linescan X-ray Agricultural Inspection

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Grant 98-35503-6340; $180,000; 3 Years

This is a collaborative research effort between the USDA Agricultural Research Service (ARS) Albany (Dr. Thomas F. Schatzki) and Carnegie Mellon University (CMU) (Prof. David Casasent) to develop classifiers for USDA agricultural inspection. The mission-oriented application considered is real-time X-ray inspection of pistachio nuts for insect feeding and damage, and other minor and serious kernel damage; this is necessary to place the product in the various Federal Grade standards. ARS and CMU have shown that X-ray data can locate insect infestations associated with aflatoxin, feeding damage, etc. that cannot be determined by present non-destructive methods.

New classifiers are considered that produce nonlinear decision surfaces in real-time. They overcome many classifier problems and have various novel properties including handling multiple clusters per class, providing low false positive rates (to avoid losing much of the good crop) and moderate false negative rates (to reduce infested nuts and aflatoxin levels). Research will determine if present real-time linescan X-ray data can provide sufficient reduction of infested nuts while rejecting only very little of the good crop and if blemish levels etc. can be determined.

The new classifiers are expected to be generally applicable to other agricultural inspection cases. This work will advance image classification in general and should greatly enhance agricultural inspection and the safety, quality, and competitiveness of U.S. agricultural products.

9801181 Modification of Cottonseed Fatty Acid Composition

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Grant 98-35503-6339; $130,000; 2 Years

Recent research indicates that it is possible to engineer changes in seed oil composition without adverse effects on crop performance (Ohlrogge, J.B., Plant Physiol. 104: 821-26. 1994). These capabilities suggest that selective modification of enzyme activities involved in fatty acid metabolism in cottonseeds will lead to newly-designed cottonseed oils. Currently, refined cottonseed oil contains an abundance of polyunsaturated fatty acid (58%, nearly all linoleic acid), a relatively high percentage of saturated fatty acid (22%, nearly all palmitic acid), and a modest amount of the monounsaturate, oleic acid (15%). The metabolic relationship between palmitic, oleic, and linoleic acid biosynthesis makes possible the manipulation of the relative percentages of these fatty acids in cottonseed triacylglycerols. We plan to alter the seed-specific expression of fatty acid metabolizing enzymes in transgenic cotton to redesign fatty acid metabolism toward the production of a new family of seed oils (high palmitic, high oleic, and low saturates, in addition to the standard cottonseed oil). Commercial cultivars that possess a significant share within the U.S. cottonbelt are targeted for introduction of novel fatty acid modifications. Results of this research will improve the overall quality of cottonseed and provide increased flexibility to cotton growers.

NON-FOOD CHARACTERIZATION/PROCESS/PRODUCT RESEARCH

Panel Manager - Dr. Douglas E. Dennis, James Madison University
Program Director - Mr. Jeffery L. Conrad

Agricultural commodities can provide the raw materials for production of numerous industrial and consumer products such as lubricants, fuels, paints, detergents, biodegradable polymers, textile fibers, fiber composites, pharmaceuticals, and various other commodity and specialty chemicals. The Non-Food Characterization/ Process/Product Research program supports research on improved methods for producing existing non-food, agriculturally-derived products and on developing new, non-food uses for agricultural commodities. Research seeks to better understand properties of agricultural materials related to their quality, value, and processing characteristics and to develop innovative products and processes for conversion of agricultural materials to non-food products.

This program also supports biofuels research directed toward understanding and overcoming factors which limit the technical and economic efficiency of production of alcohol fuels and biodiesel. Supported research focuses on the physiological, microbiological, biochemical, and genetic processes and mechanisms controlling the biological conversion of agriculturally important biomass material to alcohol fuels.

9801230 Controlled Degradation of Organic Wastes for the Production of Disease Suppressive Soil Amendments
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New Investigator Award; Grant 98-35504-6592; $63,614; 2 Years

Disposal of agricultural and food processing residues is of great concern in California, where legislation requires 50% of all organic material to be diverted from landfills by the year 2000. The agricultural industry is also facing increasing public demand to reduce pesticide use. Economic alternatives to agricultural residue disposal and sustainable methods for controlling plant diseases are imperative for the protection of natural resources. Utilization of compost as a cultivation system and carrier for biological control agents offers opportunities for both the recycling of organic wastes and reduction in pesticide application. Understanding the growth of biological control agents in compost is critical to the success of this application. The primary objective of the proposed work is to develop a process for the inoculation and growth of the biological control agent *Trichoderma harzianum* in compost. The objective will be accomplished by performing composting and inoculation experiments in a bioreactor under controlled conditions of moisture and aeration. Inoculation time will be varied. Physical, chemical and biological parameters, such as oxygen consumption and microbial community composition, will be monitored throughout the studies. This work will impact the control and management of composting processes and cultivation of composts with biological control agents by applying the knowledge gained through process experimentation and analysis. It will also affect the economics of agricultural waste management by providing an economic incentive for organic recycling.

9801326 Targeting Cellulases to Cellular Compartments in Plants for Biofuels Production
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Grant 98-35505-6964; $60,000; 1 Year

The long-term goal of this project is to develop an economical means of converting plant biomass (cellulose) to clean-burning renewable fuels, such as ethanol. We propose to generate transgenic plants that will produce useful quantities of thermostable microbial cellulases for this process. The enzymes will be inactive during growth of the plants but can be activated after harvest as a crude enzyme preparation suitable for industrial application. If successful, this strategy will reduce the cost of biomass conversion and make the production of biofuels more economical. Increased use of biofuels will create a demand for bioenergy plants such as switchgrass and alfalfa that can thrive on marginal farmland, and it will provide new uses for waste agricultural products. At the same time, promoting alternative fuels from renewable resources addresses problems associated with petroleum based fuels: air and water pollution, global warming, and U.S. dependence on an uncertain source of energy.

Our specific goals are to test the feasibility of expressing a thermostable cellulase (El) in two intracellular compartments of plants: the endoplasmic reticulum (ER) and vacuole. Sequestering the enzyme within compartments will prevent access of the enzyme to the cell wall, which contains cellulose. We will develop plasmid vehicles to target a reporter protein, green fluorescent protein (GFP), and the E1 enzyme to each compartment. We will test the protein targeting strategies by transforming tobacco BY-2 cells with the GFP cassette and determining localization of the reporter protein by microscopy. We will transform both BY-2 cells and *Arabidopsis thaliana* plants with the E1 cassettes and will compare recoverable enzymatic activity in the two target compartments.

9801567 Commercially Viable Guayule Elastomers and Latex Rubber

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Grant 98-35504-6329; $120,000; 2 Years

Although many varieties of plants are known to produce rubber, the guayule shrub, *Perthenium argentatum grey*, is the only domestic source of natural rubber that has the potential for producing enough rubber to become commercially viable. To achieve this, however, physical and mechanical properties approaching those obtained from hevea rubber, *Hevea brasiliensis*, must be achievable. Two approaches are proposed herein which exploit the inherent features of guayule rubber, thereby enabling superior performance to be attained.

The first approach utilizes a double network (DN) architecture to develop guayule rubber-based elastomers with mechanical properties superior to those of hevea rubber. We had previously determined that GR has a higher propensity for strain-induced crystallization than the purer grades of hevea natural rubber. This finding, along with the increased orientation of the DN, can enhance the failure properties of GR. Modulus, tensile strength, tear resistance, fatigue life are the focal points of the proposed research. The second approach of this research addresses the relevance of intrinsic flaws to the barrier performance of GR. Our recent experiments demonstrate directly the ability of submicron (i.e., viral-sized) particles to pass through ostensibly intact latex rubber. The quality of coalescence is known to have a definite influence on the final properties of hevea-based latex films. We will investigate the degree to which this permeability in GR reflects interstitial pathways arising during latex processing, rather than being due to intrinsic flaws. A principle objective is to determine if the barrier performance of guayule rubber is fundamentally different from that of conventional (*Hevea Brasiliensis*) latex rubbers, with an aim to assessing the potential of guayule latex based products.

9801467 High Yield Production of Specialty Proteins in Tobacco

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Grant 98-35505-6752; $174,000; 3 Years

Tobacco is a high value crop that sustains the economy in some regions of the U.S.. However, the future of tobacco is uncertain. Development of extended uses for this crop through value-added products could maintain the economic viability of these regions. One technology that already is making progress in this area is the use of virus-based vectors to produce specialty proteins in plants. Our laboratory developed the first generation of agronomically useful virus-based vectors. Presently, one company is using this technology to produce commercial products from grower-produced tobacco that is processed in a new factory in Kentucky. The capability of the virus vector technology needs to be expanded to produce a wider spectrum of products in tobacco and to have a greater impact. The objective of this proposal is to develop a new generation of virus-based vectors that overcome the current limitations on the size of proteins that can be effectively produced in tobacco and the instability of the foreign gene sequences within the vector. Our preliminary data indicate that a new approach to vector development, that divides the vector among two or more RNAs, allows designs that will solve the problems of size and instability. We have shown that this concept can work, but at this time the levels of protein production are too low for most commercial applications. We need to optimize the new multi-component vectors, as we previously optimized the current "state-of-the-art" single-component vectors. We believe that the multi-component vectors will expand the possibilities of producing high yields of specialty proteins in tobacco.

9801555 Engineering Bacteria for Fuel Ethanol Production

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Grant 98-35504-6177; $179,200; 3 Years

In previous research, we have genetically engineered bacteria for the production of fuel ethanol from sugars which are present in the polymers of woody biomass residues. These biocatalysts require that all polymers be chemically or enzymatically broken down into a mixture of simple sugars prior to fermentation. Cooking at modest temperatures with dilute acid works well as a method for converting hemicellulose into a sugar syrup, exposing the resistant cellulose fibers for further treatment. Enzymes are the preferred method to convert cellulose into soluble sugars and have the potential to produce 2-fold higher sugar yields under environmentally benign conditions. However, the cost of cellulase enzymes is currently prohibitive for commercialization. The proposed research is focussed on reducing the amount cellulase enzymes required for ethanol production from cellulase. Our approach is to further engineer the bacterial biocatalysts by adding genes which assist in the degradation of cellulose and to optimize the production of useful enzymes. These cellulase genes are being isolated from other bacteria and will be inserted into the chromosome to create stable organisms for use as industrial biocatalysts.

9801529 Effect of Corn Fractionation on Ethanol Fermentation and Coproduct Quality

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Grant 98-35504-6372; $120,300; 2 Years

The simplest method to make alcohol from corn is to grind the corn up, cook it and put all of the corn into the fermentor. Although simple, this process does not separate out the non-fermentable kernel parts before grinding. An inexpensive process has been developed to recover the germ, which is nonfermentable and full of valuable corn oil for use in ethanol production. The ethanol industry is interested in the process but has several concerns including the effect of germ removal on the DDG (non-fermentable material left after ethanol is removed). Germ removal may also effect fermentor foaming and evaporator fouling. The proposed research is to prepare samples of corn using the conventional grind (control), removing the germ, and removing both the germ and fiber. These samples will then be fermented to ethanol in a controlled fermentor and the extent of foaming measured. The beer will be centrifuged and the DDG will be dried. The ethanol will be removed from the thin stillage and the thin stillage will be tested for rate of fouling using a fouling probe developed by scientists at Argon laboratories. Excess thin stillage will be evaporated. Animal feeding trials will be performed on the DDG and thin stillage.

9801551 Characterization of *Clostridium beijerinckii* hyper-butanol producing mutant

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Grant 96-35500-3247; $180,00; 3 Years

Recent modeling studies funded by the National Corn Growers Association have indicated that the use of the *Clostridium beijerinckii* BA101 hyper-solvent producing strain in combination with either distillative or pervaporative solvent recovery systems and Corn Steep Liquor plus glucose or starch as the substrate is an approach which is competitive to the petrochemical method for producing the solvents, acetone and butanol. However, since the *C. beijerinckii* BA101 strain was produced using chemical mutagenesis, the molecular basis for why this strain produces enhanced levels of acetone and butanol remains as yet unclear. The overall objectives of this project include: (1) an examination of the molecular basis for enhanced solvent production and stability by *C. beijerincki* following addition of acetate into P-2 medium, (2) examination of genomic sequence level alterations between the wild type *C. beijerinckii* 8052 and the BA101 hyper-solvent producing mutant, and (3) examination of sugar transport and utilization in *C. beijerinckii* BA101 and 8052 wild type strains. The objectives outlined in this proposal represent a multi-faceted approach for understanding the molecular and physiological basis for the interesting changes observed in the *C. beijerinckii* BA101 strain. An understanding of the underlying basis for enhanced solvent production by the *C. beijerinckii* BA101 strain has important implications for further improvement and metabolic engineering of this microorganism for eventual commercialization of the acetone-butanol-ethanol process.

9801233 Recovering Plant Phenols by Adsorptive Separations

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Strengthening Award; Grant 98-35504-6357; $133,000; 3 Years

The conversion of petroleum-based raw materials into oxygen-containing aromatic chemicals typically requires hazardous reagents and generates substantial wastes. These problems are providing new incentives to recover "preformed" aromatics from plant extracts or from partially degraded lignin wastes generated in the pulp and paper industry. However, to separate individual chemicals from the myriad of plant phenolics will require developments in separations.

We have identified a polymeric sorbent which is capable of selectively adsorbing polar aromatics based on differences in polar interactions (e.g., hydrogen bonding). The goal of this proposed study is to develop an experimental approach to directly study the adsorptive interaction mechanisms, and to expand our studies to characterize the adsorption of complex plant phenols. Quantitatively, we propose a "Chemical Modeling" framework to analyze the results and will test predictions from this model by performing separations experiments. The significance of this proposed study is that it will experimentally demonstrate how aromatics could be commercially separated from renewable natural resources. Also, this work will provide the quantitative basis to guide the industrial development of adsorptive separations.

9801464 Environmentally Compatible Synthesis of Value-Added Chemicals from D-Glucose

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Grant 98-35504-6189; $188,000; 3 Years

Microbes are to be genetically modified to convert plant-derived glucose into p-hydroxybenzoic acid and vanillin under fermentor conditions. Both of these chemicals are currently synthetically derived from petroleum feedstocks employing toxic starting materials and intermediates. Beyond the importance of developing syntheses to value-added products, these conversions constitute unique challenges in biocatalysis. p-Hydroxybenzoic acid, which already is widely used in its esterified form in preservative and

antimicrobial applications, may ultimately be the key to commercialization of an important new class of polymers. Direct biocatalytic conversion of glucose into p-hydroxybenzoic acid requires isolation of a mutant microbe resistant to the toxicity of p-hydroxybenzoic acid. Another route proceeds through shikimic acid intermediacy. This prohibitively expensive acid has emerged as an essential starting material in the synthesis of an important new class of anti-influenza drugs. Vanillin is one of the most important additives in the food and fragrance industry. Its synthesis demands a drastic perturbation in cellular methyl group transfer reactions without compromising the viability of the host microbe. Challenges in gene isolation, enzyme over expression, and molecular evolution of enzyme function are also represented in the synthetic routes to be elaborated.

9801679 Production of Industrially Useful Monoenoic Fatty Acids in Transgenic Plants

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Grant 98-35504-6190; $165,000; 3 Years

Plant oils are a major commodity with an annual value of over $40 billion. Human consumption of vegetable oils has gradually increased and replaced animal fats to the extent that plant oils represent approximately 15-20 percent of calories consumed in American diets. Additionally, plant fatty acids have been extensively exploited for industrial uses such as lubricants, plasticisers and surfactants. In fact, approximately one third of vegetable oils produced in the world are used for non-food purposes. Using native plant genes from species which produce novel monoenoic fatty acids (e.g., *Thunbergia alata* and *Pelargonium xhortorum*), we are attempting to create new, higher-value oilseed varieties. We have previously identified novel acyl-ACP desaturase genes, each capable of synthesizing distinct monoenoic fatty acids. Our recent studies have identified additional genes that code for ACP and ferredoxin; these may be required to reach industrially useful levels of monoenoic fatty acids (50-80%) in transgenic plants. To produce oilseed crops with high levels of these fatty acids, we will create transgenic oilseed lines expressing acyl-ACP desaturases in addition to specific ACP and ferredoxin genes. We envision three broad uses for oils produced in transgenic lines expressing these genes: (1) low temperature lubricants, (2) feedstocks for polymer production and (3) production of margarine and shortenings with low saturated fats and no trans-fatty acids. Our work with novel acyl-ACP desaturase genes provides an opportunity to further increase and expand the usefulness of oil seed crops as well as open new markets for vegetable oil to be used as replacements for petroleum based oils.

9801220 Condensed-Phase Hydrogenation of Crop-Derived Organic Acids

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Grant 98-35504-6356; $96,603; 2 Years

Organic acids produced by fermentation of crop-derived starches or sugars are promising feedstocks for chemical production. One method that uses these feed materials is via hydrogenation, in which the acid reacts with hydrogen in the presence of a metal catalyst. In traditional petrochemicals production, hydrogenation takes place in the vapor-phase. This is not possible for many biomass-based feedstocks because they lack sufficient volatility, but the reaction can be carried out in aqueous solution. This project investigates aqueous-phase hydrogenation of organic acids to produce high-volume commodity chemicals, with a focus on the potentially commercial route of converting lactic acid to propylene glycol. Propylene glycol is used in consumer products and as environmentally friendly antifreeze; it is produced at a rate of nearly one billion lb/yr in the United States Research emphasis is placed on (1) identifying catalysts and reaction conditions for achieving high yields to the desired product in liquid-phase reactions and (2) gaining an understanding of the fundamental reaction steps and role of the catalyst in aqueous solution. The products formed via acid hydrogenation are excellent value-added targets for U.S. agriculture; for instance, propylene glycol produced from one bushel of corn ($2-$4) is worth $13-$15 at its current market price. In addition to developing economically attractive conversion pathways, this research will elucidate fundamental aspects of aqueous-phase hydrogenation which will broadly enhance the development of biomass refining schemes for chemicals production.

9801179 Production of Canine Oral Papilloma Virus (Copy) Vaccine in Tobacco

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Grant 98-35504-6179; $179,000; 3 Years

Tobacco is an extremely important farm commodity, producing much higher return on investment than most other crops. However, changing market economics make it prudent either to find new uses for the commodity or to find alternative crops to augment grower profits. Production of high-value proteins in genetically engineered tobacco offers an important alternative to traditional uses for the crop, while maintaining an exceptional rate of return to growers. In addition to enhancing the farm economy, this technology could also help to satisfy some of the growing need for vaccines and other therapeutic proteins. The ability to generate large quantities of such products at minimal cost would be especially welcome in veterinary applications, where demand is high but cost is a major consideration.

In this project, we will use genetically altered tobacco plants to produce a vaccine against COPY, a troublesome disease in dogs. We will also develop a purification system for the economical recovery of pure proteins from plant extracts, ensuring product quality and

enhancing profitability. We will also carry out, in a separate project, an immunization study in dogs to prove the safety and efficacy of vaccines produced by this process.

The system developed in the course of this study could be used to produce a broad array of other valuable proteins, including vaccines against important human diseases. For example, the system could be used with virtually no modification to produce a vaccine against HPV, a virus that causes cervical cancer in humans.

9801211 Investigation of the Behavior of Cellulases and their Domains in Heterogeneous Reaction Systems

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Grant 98-35504-6176; $120,000; 2 Years

Cellulose is the most abundant carbon source in the biosphere and it represents a large fraction of the biomass produced by agriculture. It also makes up to 40% of the municipal solid waste stream produced across the country. The availability and low cost of cellulose makes it an attractive raw material for industrial uses, such as energy and paper production. For the last twenty years, there has been considerable interest in the use of cellulose for the production of industrial chemicals through biological conversion processes. Biological conversion processes are of particular interest because of the many products that can be produced by fermentation and because these processes tend to be environmentally benign.

Cellulases are enzymes that are responsible for the biological conversion of cellulose to fermentable sugars. There are a variety of fungal and bacterial cellulase sources, and all of these sources contain multiple cellulases. Cellulases are composed of multiple domains which can interact with cellulose. Since many of the industrial applications of cellulases involve insoluble substrates, I am interested in how the different catalytic domains and cellulose binding domains react with the surface of an insoluble cellulose particle, and how the natural assemblage of these domains in the native enzyme influences the binding of a cellulase to cellulose and its subsequent hydrolysis.

9801232 Nanocrystalline Reinforcing Agents from Sugar Refining Waste Products

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Strengthening Award; Grant 98-35504-6358; $86,926; 2 Years

Reinforcement with rigid particles has been used for centuries to improve the mechanical strength of materials, e.g., adobe bricks, fiberglass and automobile tires. In modern glass-reinforced thermoplastic composites, where the rigid component may constitute 40% of the total weight, a critical performance factor is the adhesion between matrix component and particle surface. Recently, it has been shown that use of a highly asymmetric nanoscale particulate component at loadings of only 3-6 % by weight can generate equivalent or superior improvements in mechanical properties.

This project will expand our seed grant study into a new area, the use of individual cellulose microfibrils and microcrystals as the rigid component in nanocomposite materials. Cellulose microfibrils are rigid single crystals 5-90 nm across and 200 to 1000 nm long. Our fundamental objective is to test the suitability of sugar cane, sugar beet, other agro-waste products, and bacterial cellulose as sources of such crystals. Secondly, we seek to demonstrate that adhesion of matrix to particle can be significantly improved by topochemical modification of the cellulose crystallites. The surfaces of microcrystals, prepared from dispersed native cellulose by mild acid hydrolysis, will be derivatized to organic ester and silyl ether forms, leaving the internal crystal structure unchanged. Mechanical and degradative behavior of materials containing these compatible nanoparticles will be compared with composites using cellulose or glass fiber and with the behavior of the thermoplastic itself.

This technique can create new markets for bagasse derived cellulose as an alternative to glass or costly, synthetic polymer reinforcing agents e.g., carbon fiber and Kevlar while also leading to more environmentally benign materials.

9801465 Efficacy of Nisin as a Surface-Active Agent in Pharmaceutical Applications

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Grant 98-35504-6178; $169,400; 3 Years

We propose to test the performance of the antimicrobial peptide nisin in various pharmaceutical applications. Nisin is a small protein that can kill certain bacteria; it has already found application as a “food grade” preservative in the food and beverage industries. Nisin’s performance in pharmaceutical applications will be evaluated with reference to its ability to enhance the solubility of low-solubility drugs, and its ability to enhance drug uptake by cells. Knowledge of the effect of other proteins and surface-active agents on nisin’s performance in these areas would be critical to any drug formulation strategy, and this will be addressed as well. In particular, a technique called total internal reflection fluorescence will be used to measure the functional behavior of nisin alone and in the presence of other components, as well as at the oil-water interface. This will allow us to make predictions concerning nisin functionality in drug formulation. We will also evaluate the resistance of nisin-containing formulations to bacterial contamination, and using an animal model, nisin’s ability to promote drug absorption through mucosal cells without causing significant damage to the cells. The significance

of these issues to human health is clear, but any progress in refining and enhancing pharmaceutical formulation and administration should directly benefit the animal husbandry sector of agriculture as well. Moreover, nisin is produced by the cheese fermentation bacterium *Lactococcus lactis*. It is produced commercially from skim milk and whey by-products and thus constitutes an added-value agricultural product with a broad spectrum of promising antimicrobial and surfactant applications in agriculture.

9801226 Regulation of Respiratory and Fermentative Metabolism in Pichia

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Grant 98-35504-6966; $121,000, 2 Years

The objective of this research is to improve the value of agricultural and silvicultural residues by converting them to liquid fuels and chemicals. Better technology for fermenting xylose will increase the value of hemicellulosic sugars in agricultural and silvicultural byproducts. The research will increase ethanol production by improving our understanding of regulatory mechanisms for xylose fermentations in yeasts. It uses a genetic approach. Recent research has shown that in addition to the well studied cytochrome respiratory system, the xylose-fermenting yeast, *P. stipitis*, possesses an alternative respiratory system that is apparently involved in xylose metabolism. This respiratory system appears to reduce the efficiency of fermentation. The alternative respiratory system also appears to be induced by xylose. We want to know whether this system is essential for xylose metabolism. If not, we would seek to eliminate it by genetic manipulation, and if it were essential, we would try to attenuate its function. The research conducted under this proposal will clone, sequence and disrupt genes for alternative respiratory processes; it will determine how aeration and carbon source affect regulation of this system. The research will try to discover new regulated genes related to the alternative oxidase system, and it will create reporter constructs to follow their expression. The successful completion of this work will be applied to strain development for the fermentation of agricultural residues from renewable resources.

9801562 Production of Cellulases in Transgenic Alfalfa for use in Biomass Conversion

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University of Wisconsin, Madison; UW Biotechnology Center; Madison, WI 53706
Grant 98-35504-6341; $118,400; 2 Years

This research project is a multidisciplinary feasibility study on producing industrially important enzymes using alfalfa fields as "factories." The overall goals of the research are to develop genetically engineered alfalfa that produces high levels of industrially important enzymes and to develop the technology needed to extract these enzymes from alfalfa juice. Thus, this proposal attempts to use biotechnology to develop new non-food products from existing agricultural resources. The production of value-added crops will aid rural economies and could lead to a reduction in federal commodity support. Technology developed for extraction of these enzymes could be used to extract other value-added products from alfalfa (such as tannins and saponins) and other field grown crops. Furthermore, the availability of large amounts of enzymes will fuel industries such as biopulping, biomass conversion and bioremediation.

Specifically, this research will determine if cellulases can be produced in transgenic alfalfa. The largest single market for cellulases is the production of ethanol from waste lignocellulose. An abundant inexpensive supply of cellulases would have a major impact on the entire biofuels industry. Expression of cellulases in alfalfa may make alfalfa fiber more suitable for use as a feedstock in biomass conversion. Inexpensive supplies of ethanol could replace or reduce the use of gasoline. This would reduce the nation's strategic vulnerability and lower trade deficits for imports. There are also beneficial environmental effects. Automobile emissions are improved by burning ethanol or a mixture of gasoline and alcohol, and producing alcohol from sustainable crops does not contribute to the accumulation of carbon dioxide in the environment.

IMPROVED UTILIZATION OF WOOD AND WOOD FIBER

Panel Manager - Dr.Stephen M. Shaler, University of Maine
Program Director - Dr. Anne H. Datko

Improved wood utilization practices depend upon a continually advancing scientific foundation of basic research in wood properties and fundamental components of wood science. This program area supports research that addresses critical barriers to improved wood utilization and that will provide the scientific base from which new research and development can proceed. The major areas of focus include: (1) wood chemistry and biochemistry, (2) physical and mechanical properties of wood and basic wood processing technology, (3) structural wood engineering, and (4) forest engineering research. Innovative approaches to solving fundamental problems in the field of wood science and technology are encouraged

9802636 Fungal Delignification of Wood Chips for Kraft Pulping
Akhtar, M; Scott, G.M.
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Grant 98-35504-6986; $81,820; 2 Years

In the United States, about 85% of the pulp is produced by the kraft pulping process. This process produces paper with very high strength. However, the process has the disadvantages of being capital-and energy-intensive, giving low yields, producing troublesome waste products, and producing byproducts that are of relatively low value. The pretreatment of wood chips with a selective lignin-degrading fungus prior to kraft pulping process (bio-kraft pulping) might ameliorate some of these problems. In the proposed research, the best biopulping fungus will be evaluated on loblolly pine as it is the most commonly used wood species in the United States for kraft pulping. The fungal pretreatment and the kraft pulping conditions will be optimized. A process design that is feasible from an engineering and economic standpoint will also be the outcome of the proposed research. The fungal pretreatment causes swelling and loosening of cell wall structures which increases the porosity of wood chips. Also, the fungus modifies lignin in wood cell walls which might more easily be removed during kraft pulping. These fungus-induced physico-chemical changes in cell walls might improve chemical penetration and subsequently aid the kraft pulping processes. If so, this will reduce chemical load, cooking temperature, cooking time, emissions, and effluent load during pulping. The proposed research is directly relevant to long-range improvements in sustainability of U.S. agriculture because it will advance our understating of the interaction of biological, physical and chemical treatments of wood being used to make high quality pulps.

9802633 Is Wood Quality Influenced by the Expression of Genes Controlling Ethylene Biosynthesis?
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Grant 98-35103-6534; $179,704; 3 Years

Increased levels of compression and juvenile wood are found in the fast-grown, short-rotation trees that forest products industries are increasingly having to rely upon as cutting in old-growth forests is reduced. These lower quality woods reduce the productivity and competitiveness of industry when substituted directly for higher quality timber. Silvicultural practices can reduce to some extent the content of compression and juvenile wood in trees from managed stands; however, there is a genetic component that governs the formation of these lower quality woods. Previous studies have suggested that production of the hormone ethylene in trees is associated with compression wood formation. This project will examine the proposition that ethylene biosynthesis is a key regulator of compression and juvenile wood formation and will attempt to determine whether manipulation of the genes regulating ethylene production might afford new ways of influencing wood quality. We will clone from loblolly pine the two key genes (ACC synthase and ACC oxidase) that regulate ethylene biosynthesis and study how they are expressed in different parts of the tree and whether their expression is correlated with compression wood formation. Demonstration that these genes influence compression and juvenile wood formation would provide a logical underpinning for improved silvicultural practices, as well as provide a basis from which improved trees can be bred or genetically engineered.

9802645 A Smart Sensor Module for a Transverse Board Defect Scanner
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Grant 98-35103-6912; $54,184; 2 Years

We propose a novel microprocessor based sensing technique and architecture for building a low-cost transverse board scanner for detecting surface defects such as knots and holes. The principle behind the technique is based on the fact that light is reflected more along the grain than perpendicular to it. A transverse board scanner when implemented would consist of one sensor module every inch along the length of the board. Each microprocessor based sensor module would collect data at that location and transmit it to the host

along a common 2-wire communication bus. The differential light reflection is measured at each point by employing a low cost microprocessor coupled to a digital light sensor and two solid state light sources (LED). As the board moves transversely, each sensor module would produce data every 0.1" along the width. An experimental scanner with a scanning window of 24" would be constructed that would be able to scan a 24" long board moving transversely. The experimental scanner would have a resolution of 0.5" along the length and 0.1" along the width. The performance of the scanner would be evaluated for defect recognition accuracy. The proposed project would enable the development of smart sensors that could be eventually used in surface board scanners that could be installed in hardwood sawmills for grading applications or in a dimension mill for optimizing the cutting decisions. Successful implementation of the proposed project would, thus, result in improved yield and profitability to the sawmill industry and increase their global competitiveness.

9802918 Stabilization of High-Yield Pulp

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Grant 98-35103-6532; $146,000; 2 Years

Most chemical methods of converting wood to pulp for paper manufacture eliminate the lignin component of wood and, therefore, give low yields (40-50%). High-yield pulps that retain the lignin have the undesirable property of darkening upon exposure to light, limiting their use to low quality papers. Alleviating this problem would be a significant contribution to improved utilization of wood and wood fiber. This study continues efforts to understand the manner in which certain sulfur compounds delay the onset of darkening. Unfortunately these compounds are malodorous as well as expensive. By working with model substances and computer models to discover the molecular level mechanisms of action of these substances, it may be possible to design pulp additives having more desirable olfactory properties that will make high-yield pulp more widely useful. The project includes extensive investigations of the interaction of lignin-related structures with light and with oxidation inhibitors. Computer modeling of the highly reactive intermediates in these processes can eliminate some of the more difficult laboratory manipulations as well as provide data that are not experimentally accessible by any means. Lessons learned from the model ("test tube") systems then will be extended to systems involving actual pulp and paper.

9802710 Influence of Copolymer Architecture on the Wood Fiber/Polymer Interface

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Grant 98-35103-6556; $98,000; 2 Years.

The development of wood-fiber-reinforced plastics continues to receive high priority as a long-term goal in wood products research. In part, this priority stems from the growing resource present in the form of virgin fiber from low value hardwoods and recycled fiber from paper or pallets that is, in many respects, ideally suited for wood-reinforced plastics. Characterized by light weight, high strength, and relative ease of manufacture, this natural fiber offers a number of advantages over currently used reinforcing fibers. A notable shortcoming in wood fiber/meltable plastic (wood/thermoplastic) systems is the poor bonding between the wood and plastic. This is due to their dissimilar chemical nature, i.e., the wood surface is hydrophilic (or water-loving) while plastics are generally hydrophobic (or oil-loving). If the plastic does not form crystals (such as in polystyrene) upon solidification, its primary source of cohesive strength is entanglement of the molecular polymer chains. All these molecular considerations would have to be addressed along with efficient manufacturing and environmentally responsible methods. Our prior work has shown the potential to solve these problems, and this project is aimed at addressing what we believe is a critical hurdle - the understanding of relevant structure-to-property aspects of wood fiber/polystyrene interfaces, as applied to thermoplastics/wood composites. Its novelty is the use of newly designed long-chain detergent-type molecules (polystyrene-poly(acrylic acid-co-styrene) block copolymers) that can be delivered on wood fiber surfaces with water and, upon drying, render the wood a hydrophobic polystyrene-rich surface.

9802700 Cloning and Characterization of Homeotic Genes That Control Wood Formation in Tree

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Grant 98-35103-6533; $145,219; 3 Years

Wood is a complex plant tissue formed by a variety of different cell types organized within an extracellular matrix composed primarily of cellulose and lignin. The differentiation and organization of cells within woody tissues as well as the formation of the extracellular matrix result from the coordinated expression of many genes. The primary goal of this proposal is to identify and characterize the genes that control and coordinate the expression of genes responsible for the differentiation and development of woody tissues in aspen trees. In plants, a family of genes known as MADS-box genes has been shown to control the expression of genes responsible for the differentiation and development of a wide variety of plant tissues and organs. We propose to identify MADS-box genes that are likely to have a regulatory role in the development of wood, characterize the expression patterns of these MADS-box genes during wood formation, and begin studies to analyze the function of these MADS-box genes by altering their level of expression in

transgenic aspen. Eventually, it is hoped that these genes may be used to modify tree growth for increased wood production or improved wood quality.

9802720 The First Generations of True Lignin-Based Plastics

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Annually in the United States, huge quantities of kraft lignins are generated as byproducts from the chemical conversion of wood chips into pulp for making paper. Traditionally these industrial byproduct lignins have been burned in the recovery furnaces of pulp mills. However, maximizing production has led, in many instances, to a level of operational congestion that creates a surplus of kraft lignin which is difficult to use. The more such circumstances proliferate, the more inefficiently are wood resources utilized in manufacturing pulp and the greater are the costs of preventing pollution from pulp mills. The present research project proposes to establish a foundation for creating a broad range of plastics that are fundamentally based on simple industrial byproduct lignin derivatives. The work will focus upon the identification of plasticizers that function by interacting preferentially with the lignin components so as to increase intermolecular separation and, thus, enhance the mobility of the polymeric chains in the solid state. Industrial jack pine kraft lignin will be purified and fractionated by ultrafiltration, and the resulting samples will be chemically derivatized through straightforward alkylation and acylation reactions. Blends of the kraft lignin derivatives with promising plasticizer components will be solvent-cast into materials of suitable form for preliminary mechanical testing and thermal analysis. Dispersions in water of the lignin-based blend formulations for the most successful materials will be spray-dried to produce powders for extrusion-molding thermoplastic pellets, films and parts; these will again be subjected to thorough mechanical and thermal evaluation.

9802707 Binuclear Manganese Complex-Catalyzed Bleaching of Pulps with Hydrogen Peroxide

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Grant 98-35103-6781; $91,384; 2 Years

Bleaching of pulp is traditionally accomplished in the pulp and paper industry by bleaching sequences involving utilization of elemental chlorine and chlorine dioxide to remove residual lignin by oxidative degradation. In spite of its excellent selectivity, chlorine is largely responsible for the formation of a wide variety and large amount of chloro-organics, which are toxic because of their carcinogenicity and mutagenicity. Under the pressure of the increasing environmental demands, bleaching of pulp with inexpensive non-chlorine containing chemicals has been becoming more and more desired. A group of Mn(IV) complexes has been found recently in our laboratory to selectively catalyze oxidation of residual lignin with hydrogen peroxide. In addition, the Mn(IV) complexes are very selective and effective in catalyzing the hydrogen peroxide bleaching of softwood pulps even in trace amounts, approximately in the range of 60-70 ppm per pulp level without adverse effects on the physical properties of the pulps. All of these indicate that environmentally benign and industrially viable totally chlorine free (TCF) and elemental chlorine free (ECF) hydrogen peroxide bleaching processes can be developed by using properly selected binuclear Mn(IV) complexes as catalysts. The overall objective of the proposed research project is to explore the binuclear Mn(lV)-complex-catalyzed hydrogen peroxide bleaching of pulps. This may lead to the development of new hydrogen peroxide bleaching of pulps that are environmentally benign and industrially viable totally-chlorine-free (TCF) and elemental-chlorine-free (EFC) bleaching processes.

9802657 Affinity Ligands for Cellulolytic Enzymes

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Microbial cellulolytic enzymes, which catalyze the hydrolysis of cellulose, are currently used in wood and wood fiber bioprocessing. These cellulolytic enzyme preparations generally contain a complex mixture of enzymes. The individual enzyme components present in these enzyme preparations have unique specificities and, thus, modify wood fibers (cellulose fibrils) differently. The vast potential of using cellulolytic enzymes for the production of novel products can be realized only when industry is capable of making use of these unique specificities, such that "specialty" enzyme mixtures can be formulated. A major barrier to this end is our current lack of information on the properties of the individual cellulolytic enyzmes. An obvious prerequisite for obtaining this type of information is the ability to obtain functionally pure enzymes. The principle objective of the proposed research is to develop methods for the purification of cellulolytic enzymes. The research focuses on the development of “affinity ligands,”which in this case are low molecular weight compounds that specifically associate with cellulolytic enzymes. The affinity ligands will be chemically tethered to an insoluble support, the support will then serve to selectively adsorb cellulolytic enzymes. The extent to which a particular affinity ligand interacts with an enzyme will be dependent on the specificity of that enzyme. Three classes of affinity ligands will be tested for their usefulness in the purification of cellulolytic enzymes. All of the affinity ligands will be substrate analogs; they will differ with respect to their extent of derivitization.

9802713 Elasticity Constants of Structural Composite Lumber

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Grant 98-35103-6580; $80,095; 2 Years

Structural composite lumber (SCL) materials represent an innovative technological development for the manufacture of high performance engineered wood products. SCL manufacture involves a reconstitution process where veneer or strands of wood combined with thermosetting resin are consolidated through applied heat and pressure. The process allows high quality products to be fabricated from young growth and lower quality raw materials for more efficient utilization of forest resources. Sawn structural lumber recovers only 40% wood fiber, while 76% conversion is possible for reconstituted composite lumber. Elasticity is the fundamental property that describes how materials deform under the mechanical action of a load. Material constants for elasticity are used from the most basic design computation progressively to perform more in-depth analyses with advanced finite element modeling techniques. Because SCL materials are relatively new, limited information is available to describe their elasticity behavior. Elasticity characteristics for these orthotropic wood-based composites are sparsely documented with most information limited to the flexural modulus of elasticity (MOE). Other important elastic constants for shear modulus (G) and Poisson's ratio (v) are unknown for most SCL materials. Experimental test efforts are designed to increase knowledge on SCL material response under axial load conditions will further information on resistance to shear distortion. Elasticity properties will be studied on a range of commercial SCL products including laminated veneer lumber (LVL), parallel strand lumber (PSI) and laminated strand lumber (LSL). Study results will enable designers and engineers to more effectively optimize structural designs and enable researchers to explore other adaptive applications of SCL materials.

9802714 Improving work team performance in wood-based production facilities

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Increased competition is forcing U.S. agricultural and wood products producers to seek greater efficiency with increased reaction to marketplace demands. While plant and equipment technologies may be easily upgraded to meet demands, the critical human resources in place at production facilities are much more difficult to “upgrade” to a corresponding skill level. While many wood-based and agricultural products producers are increasingly relying on work teams to meet such challenges, team structures and design have proven difficult to initiate, maintain and manage. This project, therefore, proposes to investigate various aspects of work teams in wood-based production facilities in order to better understand how U.S. producers can utilize such teams to increase productivity and competitiveness. Data will be collected from a variety of teams at wood-based production facilities.

Research objectives will include investigations of the following: (1) how diversity in team membership can facilitate group performance on complex tasks by providing the skills and knowledge that enable the group to leverage physical resources most effectively, (2) means by which external relations with groups outside of the production teams (e.g., communications with marketing, R&D, or foresters) can impact activities and performance of the teams, (3) impacts that boundary-spanning activities of teams have on their overall effectiveness, and (4) the impacts that communication both within and between work teams has on overall performance of a team as well as on its individual members. The fundamental goal of this research is, therefore, to investigate how teams in U.S. agricultural and forest industry facilities can best be organized and managed to increase productivity and efficiency.

9802719 Automated Analysis of CT Images for the Inspection of Hardwood Logs

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Grant 98-35103-6581; $96,086; 2 years

The quality of lumber produced by hardwood sawmills depends on the quantity, type and location of defects. Thus, each log is sawn to preserve the most clear wood in resulting boards. Traditionally, a breakdown strategy is chosen by visually examining the exterior of a log and is modified as sawing exposes the logs interior. Developing nondestructive methods that can accurately detect and characterize interior defects is critical to future efficiency improvements for sawmills. Recent studies have demonstrated the feasibility of computed tomography (CT) image analysis for this purpose, and CT scanner manufacturers have begun R&D efforts to build scanners for the wood processing industry. Thus, practical CT analysis methods and software are urgently needed for hardwood log inspection. The proposed research will build on prior work to develop novel methods for CT image analysis. The major claim is that artificial neural networks can be combined with higher-level analysis methods to provide accurate results quickly. This work is of great importance to sawmill and veneer mill operators, because the resulting methods will significantly improve the quality and consistency of products obtained from increasingly scarce timber, and assure viability of rural-based mills in a global economy. In addition, improved identification of defects will reduce wasted wood, thus, preserving natural resources. These techniques will benefit other image-based inspection applications as well. This project represents a close collaboration between Virginia Tech, the responsible organization in this proposal, and the Southern Research Station of the USDA Forest Service.

9802699 Cyclic Response of Multiple Bolted Connections

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This project will develop a computer model that will predict the cyclic response of multiple-bolt connections in wood. This will allow the response of wood structures to be analyzed accurately for hurricane and earthquake loading. Previous research and modeling efforts have focused on single bolt connections, while most connections in structures are made with more than one bolt. Failures of multiple bolt connections have occurred in many materials, and it is well-known that in hard materials, such as steel, a reduction in the effective capacity of bolts occurs as the number of bolts in a connection is increased. However, wood is not a hard material and deforms around a bolt as the connection is loaded. Therefore, the possibility of load redistribution within the connection is real. A combination of computer modeling and physical testing of multiple bolt connections will be undertaken in this project in order to quantify the level of damage that occurs in the wood material, as well as the magnitude of load redistribution. Instrumented bolts that can measure the level of force in the individual bolt will be used in the experimental portion of the project to accurately quantify the changes in load distribution during cyclic displacements. The results will be used to validate the computer model. After validation, the computer model will be used to investigate the different parameters that affect the performance of the connection.

9802660 Creep and Duration-of-Load in Timber Beam-Columns

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Most compression members in a structure are subjected to bending in addition to axial loads. A common example is the top chord of a wood truss. Through truss action, it carries axial loads (typically compression). Additionally, since the top chord also directly supports the roof sheathing, it supports the roof load (e.g., snow) along its length, thus, creating bending in the member. Additional bending moment arises from the axial load acting through a lever arm equal to the transverse deflection of the member that is caused by the lateral load. This additional moment is often referred to as P-D or secondary moment, since equilibrium conditions are formulated based on the deformed structure. In wood structures, these second-order effects are compounded by the natural, viscoelastic (or creep) behavior of wood. Wood structures are subject to sustained dead, live and snow loads, and the creep response of the wood under sustained loads tends to increase deflections over time. The transverse deflections in wood beam-columns will likewise increase under sustained load due to creep, thus, increasing secondary moments. The effect of creep, however, on the combined load behavior of wood members has not been studied adequately nor has the effect been included in design checking equations. Through this proposed research, an appropriate and complete database will be developed for the long-term performance of timber beam-columns. The experimental variables considered in the proposed research include relative contribution of loading (axial vs. bending), magnitude of loading, and material (size, species, and grade). From the resulting data, suitable factors for use in design-checking equations will be developed to account for such behavior.

802659 Delignification by Laccase/Mediator Systems

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Grant 98-35103-6583; $159,329; 3 Years

There is a need for chlorine-free bleaching agents in paper manufacture because the treatment of wood pulps with chlorine generates toxic pollutants. Laccase enzymes show promise for this application because they bleach pulps in the presence of certain small mediator compounds. However, currently known mediators are too expensive for practical application. An improved knowledge of how laccase/mediator systems bleach pulps will facilitate the search for better mediators. Preliminary work shows that some mediators are able to degrade major structures in the lignin that must be removed during pulp bleaching. The reactions that occur suggest that the lignin is attacked by free radicals that are derived from the mediators. That is, the best mediators may be those that laccase oxidizes to strongly oxidizing free radicals. To test this hypothesis, a variety of potential mediators will be tested with laccase against model compounds that represent lignin structures. The models will contain chemical substituents that either favor or impede attack by free radicals. The behavior of the models in reactions with laccase and mediators will show which mediator systems have oxidation potentials that are optimal for delignification.

9802644 Relationship of MOR and MOE to X-ray Diffraction Microfibril Angle in Southern Pine

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Plantation forestry has become a significant portion of the US. agricultural industry. It is expected that much of the future timber supply will be from improved trees, both softwood and hardwood, grown on managed plantations. The short age-rotation resource will

contain higher proportions of juvenile wood which has cellular structure that is substantially different from that in past harvests. Properties of solid wood as well as existing and new composites depend on the cellular structure of the wood fibers or particles that are used in forming them. Definitive information is needed on the influence of the components of cell structure on lumber properties so that improved selection and utilization methods may be applied. The microfibril angle (MFA) of the S_2 layer is a critical component of cell structure that significantly affects the mechanical properties of these wood products. The microscopic techniques used for obtaining information on S_2 microfibril angle are very slow and tedious. A much more rapid method of determining microfibril angle must be found. This project will investigate and further develop one of the most rapid methods for determining microfibril angle. X-ray diffraction microfibril angle estimates will be made on samples from 52 loblolly pine trees from rapidly grown plantation material. These measurements will be correlated with preexisting mechanical test information from the same material.

9802665 Performance Characterization of Wood-FRP Interface Bonds

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Grant 98-35103-6579; $77,479; 2 Years

There is a world-wide need to rehabilitate and improve civil infrastructures, and new construction materials and methods are being intensely investigated to alleviate current problems and provide better and more reliable future services. The combination of wood with fiber-reinforced plastics (FRP) offers great potential for the development of hybrid materials with superior characteristics to conventional materials. New wood-FRP hybrid materials for high volume construction applications are being developed from low-cost constituents, and these materials can offer enormous benefits to the US wood industry by enhancing the performance of lower-quality and under-utilized wood species not presently used for structural components. Current research on wood reinforcement has focused on the use of FRP strips or fabrics bonded to wood with adhesives. This technology has resulted in significant increases in stiffness and strength of wood members, such as Glulam. However, there is a concern with the long-term reliable performance of the interface bond; the delamination of the FRP can lead to premature failure of reinforced members. This study will develop analytical/experimental methods to characterize the performance of wood-FRP interface bonds for delamination and strength under exposed environments and service load conditions. The results of this study will be used to qualify adhesive systems, establish service performance, obtain interface strength data useful in design, and provide fracture toughness data of the interface useful to evaluate potential in-service delamination. This research will contribute to promote the market acceptability of wood-FRP products by developing reliable methods for evaluation of durability of interface bonds.